

Please amend this application as follows:

IN THE SPECIFICATION

Please replace the paragraph at page 28, lines 11 to 23 with the following amended paragraph:

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Many adjuvants are known in the art that could be used to stimulate an immune response to peptides of the current invention. For example, alum, proteosomes, certain lipids, such as palmitic acid (see below), QS21, or ALHYDROGEL® (2%; #A1090BS, Accurate Chemical and Scientific Company, Westbury, NY) could be used as an adjuvant in the present invention (da Fonseca, D. P., et al., "Identification of new cytotoxic T-cell epitopes on the 38-kilodalton lipoglycoprotein of Mycobacterium tuberculosis by using lipopeptides," *Infect. Immun.* 66:3190 (1998); Sheikh, N. A., et al., "Generation of antigen specific CD8+ cytotoxic T cells following immunization with soluble protein formulated with novel glycoside adjuvants," *Vaccine* 17:2974 (1999); and Moore, A., et al., "The adjuvant combination monophosphoryl lipid A and QS21 switches T cell responses induced with a soluble recombinant HIV protein from Th2 to Th1," *Vaccine* 17: 2517 (1999).

Please replace the paragraph at page 35, line 21 through page 36, line 10 with the following amended paragraph:

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ELISA. Screening of hybridoma culture supernatants was done by ELISA. U-bottom microtitration plates (Costar, Cambridge, Mass.) were sensitized with 50 µl of *S. pneumoniae* whole cell suspension (10⁹ cfu/ml) diluted 1:4,000 in 0.1 M carbonate buffer, pH 9.6, and kept for 16 h at 4°C. The plates were washed 5 times with 0.9% NaCl containing 0.05% TWEEN® -20 (NaCl-T). Culture supernatants (50 µl) from the fusion plates were added to 50 µl of a solution containing 2% bovine serum albumin (BSA). 10% normal rabbit serum, 0.3% TWEEN®-20, and 0.02% Merthiolate in phosphate buffered saline (PBS), pH 7.2, (ELISA diluent, Wells et al. (1987) *J. Clin. Microbiol.* 25:516-521) in the plates and were incubated for

B₂ 30 min at 37°C. The plates were washed 5 times with NaCl-T. Fifty microliters of goat anti-mouse immunoglobulin horseradish peroxidase conjugate in ELISA diluent was added to each well. The plates were incubated for 30 min at 37°C. The plates were washed, and 50 µl of 3,3',5,5'-tetramethylbenzidine (0.1 mg/ml in 0.1M sodium acetate, 0.1 M citric acid (pH 5.7) with 0.005% hydrogen peroxide) was added to each well and incubated for 30 min at 37°C. The reaction was stopped by adding 1 ml of 4 M H₂SO₄ and the optical density was read on a Dynatech ELISA Reader (Dynatech Laboratories, Inc., Alexandria, Va.) at 450 nm. An optical density of greater than 0. 200 was considered positive.

Please replace the paragraph at page 36, line 11 through page 37, line 10 with the following amended paragraph:

B₂ SDS-PAGE and immunoblot analysis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Tsang et al. ((1983) *Methods Enzymol.*, 92:377-391), using an 8% acrylamide resolving gel. Equal volumes of sample buffer (5% SDS-10% 2-mercaptoethanol-20% glycerol in 0.01 M Tris HCl, pH 8.0) and cell suspension containing 2.4 µg protein per µl were mixed, heated at 100°C for 5 min, and a 5-µl sample was applied to 1 of 15 wells. If the final protein content of the portion of sample to be tested was <1.2 µg/µl, a volume up to 10 µl of sample was applied to achieve a final concentration of 6 µl of protein per well. Protein concentrations were determined by the method of Markwell et al. ((1978), *Anal. Biochem.* 87:206-210), with BSA as the standard. Proteins separated by SDS-PAGE were either silver stained by the method of Morrissey ((1981) *Anal. Biochem.* 117:307-310) or electroblotted onto nitrocellulose (Schleicher & Schuell, Inc., Keene, N.H.). The immunoblot procedure was done according to the method of Tsang et al. (1983) with slight modifications. The blots were given three 5-min washes with PBS, pH 7.2, containing 0.3% TWEEN®-20 and were gently agitated overnight (16 h) at 25°C. The blots were blocked for 1 h with casein-thimerosal buffer (CTB) (Kenna et al. (1985) *J. Immunol Meth.*, 85:409-419). After three rinses with CTB, the blots were exposed to goat anti-mouse immunoglobulin horseradish peroxidase conjugate (Bio-Rad Laboratories, Richmond, Calif) for 2 h at 25°C.

B3 Conjugate dilutions (1:2,000) were made in CTB. The blots were again rinsed three times with CTB and exposed to 3,3'-diaminobenzidine-4-hydrochloride in PBS, pH 7.2 (0.5 mg/ml), with 0.003% H₂O₂ for 5 min at 25°C. Reactivity was expressed as a visible colored band on the nitrocellulose paper. Low molecular-mass protein standards (Bio-Rad) were used in PAGE and immunoblotting. Rabbit antisera to the protein standards were used to develop the standards (Carlone (1986) *Anal. Biochem.* 155:89-91). Molecular masses were calculated by the method of Neville et al. ((1974), *Methods Enzymol.* 32:92-102) using appropriate molecular mass standards.

Please replace the paragraph at page 37, lines 11 to 26 with the following amended paragraph:

B4 Immunofluorescence Assays. A bacterial suspension containing approximately 400-500 cfu per field (10 µl) was allowed to dry at room temperature on each well of acetone-resistant, 12-well (5 mm diameter), glass slides (25 x 75 mm) (Cel-Line Associates, Newfield, N.J.). The slides were then immersed in acetone for 10 min and air dried at room temperature. MAbs were added to the slides, which were incubated for 30 min at 37°C. After incubation, the slides were gently rinsed with PBS and soaked twice at 5-min intervals, blotted on filter paper, and air dried at room temperature. Fluorescein-labeled rabbit anti-mouse immunoglobulin (courtesy of W. F. Bibb, CDC) was then added, and the slides were incubated for 30 min at 37°C. They were then washed twice with PBS and gently blotted on filter paper. Slides were covered with carbonate-buffered mounting fluid, pH 9.0, and cover slips and were then read with a LEITZ DIALUX® 20 fluorescence microscope equipped with a HBO-100 mercury incident light source, an I cube filter system, a 40x dry objective lens, and 6.3x binoculars (Leitz, Inc., Rockleigh, N.J.).

Please replace the paragraph at page 37, line 27 through page 38, line 25 with the following amended paragraph:

B5 Immunoelectron-microscopy. Pneumococcal cells were washed two times with PBS and fixed in a freshly made mixture of 1% paraformaldehyde-0.1% glutaraldehyde for 20 min at 4°C. The cells were dehydrated in a graded alcohol series and then in a 1:1 mixture of absolute

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ethanol and LOWICRYL® K4M (Ladd Research Industries, Inc., Burlington, Vt.) for 1 h at 4°C. The cells were pelleted and suspended in a 1:2 mixture of absolute ethanol and LOWICRYL® K4M for 1 h at 4°C. They were again pelleted and suspended in LOWICRYL® K4M (undiluted) for 16 h at 4°C. The cells were transferred to fresh and undiluted LOWICRYL® K4M two times during the next 24-hour period. The LOWICRYL® K4M-treated cells were imbedded in gelatin capsules and placed in a box lined with aluminum foil. The capsules were hardened using a short-wave UV light source (35 cm distance for 72 h at -20°C). The box was brought to room temperature, and the capsules were allowed to continue hardening for up to 14 days. Samples of the capsule were cut into 100-µm thin sections and picked up on nickel grids. Grids containing the sample were placed on a droplet of ovalbumin solution in PBS containing sodium azide (E.Y. Laboratories, Inc., San Mateo, Calif) for 5 min. The grids (wet) were transferred to a solution of primary MAbs diluted in a solution of BSA reagent (1% BSA in PBS containing 0.1% TRITON X- 100™, TWEEN® -20, and sodium azide) (E. Y. Laboratories) and incubated for 1 h at room temperature or 18 to 48 h at 4°C in a moist chamber. For antibody binding controls, other grids were wetted with MAbs against *Legionella pneumophila*. The grids were rinsed two times with PBS and incubated on droplets of goat anti-mouse IgG-labeled colloidal gold particles (20 µm)(E. Y. Laboratories) for 1 h at room temperature. The grids were rinsed two times and post-stained with osmium tetroxide, uranyl acetate, and lead citrate. The grids were examined with a Philips 410 transmission electron microscope.

Please replace the paragraph at page 57, lines 9 to 24 with the following amended paragraph:

P6
High affinity specimens from the library obtained using the procedures of Example 11 were propagated and sequenced. For each MAb, ten phage specimens resulting from the selection process were sequenced. Approximately 1 µg of single-stranded DNA was purified by phenol and chloroform extraction, ethanol precipitated and resuspended in 7 µl water.

Sequencing reactions were performed using a 27-mer primer complementary to the FUSE 5 vector sequence derived from a region in wild-type pIII common to all fd-tet derived vectors and

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³⁵S SEQUENASE® version 2 (U.S. Biochemicals, Cleveland, OH). The sequences obtained are shown in Table 4. They were compared to known sequences of PsaA strains 2 and 6B using Clusta IV and tFasta programs to identify the epitope on the PsaA with which each peptide is aligned most closely. These epitope positions are also given in Table 4. The peptide obtained using MAb's 8G12, 6F6, and 1E7 align to PsaA best when an additional residue is present on the protein where the gap appears after residue 13 of the peptide (SEQ ID NO: 7 and SEQ ID NO: 8).

Please replace the paragraph at page 60, lines 1 to 11 with the following amended paragraph:

B⁷
Anti-PsaA specific ELISA was performed as follows: Nunc immuno MaxiSorb™ plates were coated with 5 µg/ml of purified native PsaA protein at 4°C overnight. Plates were washed with PBS/Tween buffer (PBS containing 0.01% TWEEN®-20) and blocked with PBS/Tween buffer containing 1% BSA. Serial dilutions of mouse sera starting at 1:10 in PBS/Tween/BSA were incubated for 1 h at 37°C. The plates were washed four times with PBS/Tween. Anti-mouse IgG and IgM conjugated to horseradish peroxidase (Sigma, St. Louis, MO), diluted at 1:4000 in PBS/Tween/BSA, were added to the plates. Anti-PsaA antibodies were detected with o-phenylenediamine substrate for 30 min in the dark. Absorbance was read at 490 nm on Microplate E1311 (Biotek, Winooski, VT).

Please replace the paragraph at page 62, line 25 through page 63, line 21 with the following amended paragraph:

B⁸
To analyze the ability of the peptide SEQ ID NO: 5-lipidated and SEQ ID NO:5-unlipidated to protect against *S. pneumoniae* challenge, ten-week-old ND-4 mice (Swiss Webster) were immunized on a three-dose regimen. Test mice ($n = 15$ for each peptide) received an initial dose at Day 0 of 100 µg followed by booster doses at 3 and 5 weeks of 50 µg of the appropriate peptide. The peptide SEQ ID NO: 5-lipidated was suspended in 100 µl PBS 0.01 M, pH 7.2, while the unlipidated peptide SEQ ID NO: 5-unlipidated, was mixed with the adjuvant

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ALHYDROGEL® (2%; #A1090BS, Accurate Chemical and Scientific Company, Westbury, NY) at 6.3 mg/ml in PBS to enhance the immunogenicity of the peptide. Control mice ($n = 12$) were similarly immunized but without peptide. Each mouse was immunized subcutaneously between the shoulders. One week following the final boost, all mice were challenged with 4.9×10^6 cfu of *S. pneumoniae*, strain PLN-D39 (kindly provided by James Paton, Women's and Children's Hospital, North Adelaide, S.A. Australia), a pneumolysin-negative derivative of D39. This was followed 5 days later by euthanasia and culturing of nasal washes. PBS nasal washes were done by the method of Wu, H.Y., et al., ("Establishment of a *Streptococcus pneumoniae* nasopharyngeal colonization model in adult mice," *Microb. Pathog.* 23:127 (1997)). The wash was diluted 3x out to a final dilution of 1:486. Fifty microliters of each dilution was cultured on blood agar + gentamicin plates (Trypticase soy agar supplemented with 5% defibrinated sheep blood and 0.5% gentamicin). Data from NP colonization and carriage in immunized mice and placebo (PBS-immunized controls) were analyzed using either the t-test or the Mann-Whitney rank sum test. Nasopharyngeal carriage is the number of colony forming units per nose. Nasopharyngeal colonization is either positive or negative for a mouse depending on whether at least 1 cfu forms in 25 μ l of nasal wash.

IN THE CLAIMS

Please amend the claims as follows:

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1. (Amended) An isolated peptide that immunospecifically binds to a monoclonal antibody obtained in response to immunizing an animal with *Streptococcus pneumoniae* pneumococcal surface adhesion A protein (PsaA), wherein the peptide is a multiple antigenic peptide.
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11. (Amended) The peptide of claim 1, wherein the multiple antigenic peptide has at least one first arm comprising the amino acid sequence of SEQ ID NO: 5, at least one second arm comprising the amino acid sequence of SEQ ID NO:6, and at least one third arm comprising the amino acid sequence of SEQ ID NO:7.